THE RELEASE OF EFFERENT NERVE ACTIVITY IN THE ROACH, PERIPLANETA AMERICANA, BY EXTRACTS OF THE CORPUS CARDIACUM[1]

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Several attempts have been made to correlate patterns of endogenous activity as observed in isolated portions of invertebrate nervous systems with the normal behavior of the intact animal (von Holst, 1934; Roeder, 1955; and Harker, 1956 and 1958). Roeder, Tozian and Weiant (1960) found that decapitation of roaches or of mantids markedly increases the level of endogenous activity in the efferent nerves from the cercal ganglion which innervate the phallic musculature and in the efferent nerves from the metathoracic ganglion which innervate the thoracic musculature. As the activity in the phallic nerves increases, rhythmic bursts of nerve spikes are seen. These motor bursts on the several nerve branches leading to the phallomeres are not obviously coordinated, but they result in ordered, rhythmic movements of the genitalia. The behavioral significance of this endogenous nerve activity is suggested by the observation that in male mantids the copulating reflexes appear with great intensity following decapitation. Indeed, under natural conditions, the male is often beheaded by the female before he mates with her (Roeder, 1935). Control of this sexual behavior and of the efferent nerve activity in the nerves to the phallomeres and to the thoracic musculature appears to be effected through the agency of an inhibiting center in the subesophageal ganglion.

In most of these experiments (Roeder, Tozian and Weiant, 1960) there was a time lag of five to fifteen minutes between the removal of the head and the appearance of bursts of spikes in the efferent nerves. This time lag might result from a decreasing discharge of severed inhibitor neurones, augmented by injury potentials. On the other hand, it might represent the time necessary for the inactivation or removal of an inhibiting neurohormone. Such a material, if present, could be transported by proximo-distal flow down axons within the nerve cord to the site of its action.

A variety of substances are to be found in roaches which affect their behavior and some of these may be produced within the central nervous system itself (Beament, 1958; Colhoun, 1958, 1959; Smyth, 1959; and Sternberg and Kearns, 1952). The work of Özbas and Hodgson (1958) showed that the injection of corpus cardiacum extracts produced stereotyped behavior in roaches and that these extracts had a pronounced inhibitory effect on the activity in isolated roach abdominal nerve cords. Their results led us to attempt to block the inhibitory influences

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impinging on the efferent nerve cells in the cercal and metathoracic ganglia by the use of similar extracts.

Materials and Methods

Most of the observations of activity in efferent nerves to the phallomeres and in the connectives of the abdominal nerve cord were made using male *Periplaneta americana* as test animals. For these experiments the animals' wings and legs were removed and the bodies were pinned dorsal side down on a cork. Most of the abdominal contents were removed. Nerve IX or nerve X from the cercal ganglion (Fig. 1, E^2) was picked up on a small tapered silver wire hook electrode and allowed partially to dry. In many experiments afferent impulses in the nerve and the muscle potentials resulting from the efferent activity were eliminated by cutting the nerve distal to the recording electrode. The indifferent electrode was a chlorided silver wire touching the inner abdominal wall. Simultaneous recordings of nerve activity in the connectives of the abdominal cord were made by raising a connective on a pair of silver hook electrodes (Fig. 1, E^1). The input from the phallic nerve fibers was amplified with a Grass P6 preamplifier and observed on a Dumont 304A oscilloscope. The amplified signals were also recorded on one channel of a Grass Polygraph inkwriter using a 5P3 integrator preamplifier. The activity from the abdominal connectives was amplified by a 5P3 preamplifier and appeared on the other channel of the inkwriter. The two inputs could be transposed at any time so that visual observations or photographs could be made of either the nerve impulses from the cord or of those from the phallic efferent nerves. Meanwhile, a continuous record of the integrated activity from both sources appeared on the inkwriter.

Endogenous efferent nerve activity in the thoracic region was sampled from an electrode pair placed under nerve 3 (Fig. 1, E^3) from the left side of the metathoracic ganglion. The method has been described previously (Weiant, 1958). All other nerves arising from the thoracic ganglia were severed, eliminating all sensory input from these segments. The longitudinal connectives between the thoracic ganglia were left intact, as were the connections of the thoracic cord with the head ganglia and abdominal ganglia. Peripheral connections of extra-thoracic regions of the nervous system were also left intact. Activity of the internuncial fibers within the thoracic connectives was monitored simultaneously with that of nerve 3 by placing a pair of electrodes under the thoracic connectives above and below the prothoracic ganglion (Fig. 1, E^4).

Many of the same extract samples were tested for their action both on the phallic motor nerves and on the thoracic efferent fibers. In experiments with both of these preparations, injections into the head capsule of 0.01 to 0.02 ml. of extract were made as close to the subesophageal ganglion as possible. In some tests with the thoracic preparation a drop of extract was applied directly on the metathoracic ganglion; in other experiments using the abdominal preparation, several drops of extract were used to bathe the last three abdominal ganglia with their connectives. Some tests were conducted on *Periplaneta* from which the corpora cardiaca had previously been removed.

Extracts were made from the corpora cardiaca of the following species of roaches: *Blaberus craniifer, Blaberus giganteus, Periplaneta americana, Leucophaea*

maderae, Byrsotria fumigata, and *Diploptera punctata.* Extracts were from the glands of adult animals except in one case in which an extract was made from the corpora cardiaca of penultimate stage nymphs of *Periplaneta.*

The paired corpora cardiaca were excised according to the technique of Özbas and Hodgson (1958) and placed on the end of a small glass pestle. The glands were removed as quickly as possible after the donor roaches had been caught in order to minimize the loss of active material due to excitation of the roach (Hodgson, personal communication). When the requisite number of corpora cardiaca had been collected, they were triturated in a small mortar. Either Hoyle's or Pringle's insect saline was added from a hypodermic syringe to dilute the extract to the concentration desired. Pringle's proved to be the more satisfactory saline for maintaining the nerve activity in the preparations.

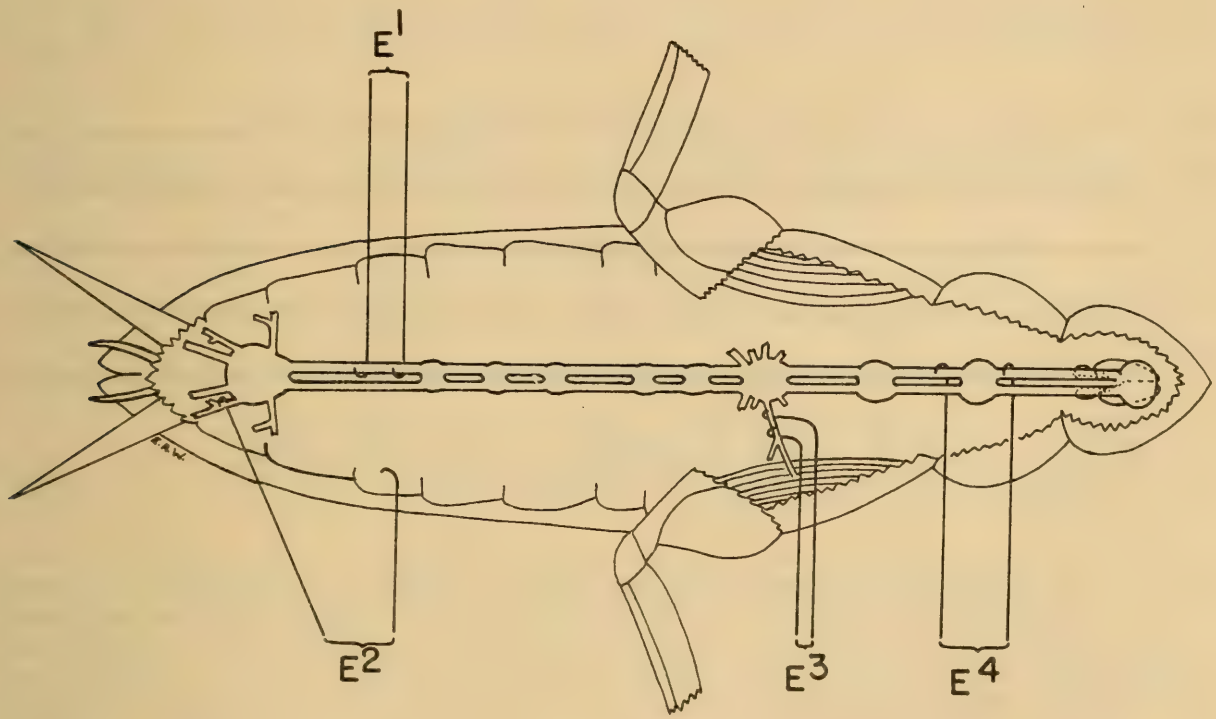

FIGURE 1. Diagram of the central nervous system of *Periplaneta americana* showing placement of electrodes.

In many of the experiments the mortar containing the extract was heated at 95° to 100° C. for five minutes. A coagulum formed in the bottom of the mortar, leaving a fairly clear solution which was used for the tests. Longer exposures to heat seemed to impair the activity of the extract, but a brief period of heating left the potency unchanged. Unheated extracts lost their activity somewhat after four to five hours at room temperature or after about ten hours at 12° to 14° C. Heating approximately doubled the time that the extracts would remain active.

Four experiments were conducted with extracts which were quick-frozen on blocks of dry ice and kept frozen for periods of twenty-four hours to a week. Two of these extracts were heated before being frozen. None of the quick-frozen extracts showed any loss of activity. This seems a promising method for preserving such material.

Results

Efferent nerve activity of the last abdominal ganglion. The effects of decapitation or of nerve cord transection on efferent fiber activity of the last abdominal ganglion have been described in detail elsewhere (Roeder, Tozian and Weiant, 1960). In a roach with the nervous system intact, spike potentials are observed in only a few efferent fibers of the nerves supplying the phallic musculature. In those fibers which are active, spikes recur regularly and at a relatively low frequency. If the animal is decapitated, there is a brief injury discharge followed by a return to the previous level of activity. After five to ten minutes the number of active fibers in the phallic nerve begins to increase, and a slowly-rising crescendo of activity is observed due to a steady increase in the spike frequency in both newly— and previously— active fibers. Typically this increased efferent discharge gradually becomes patterned into a sequence of impulse bursts. At first the bursts are isolated, but they soon become rhythmic and increase steadily in frequency for the next

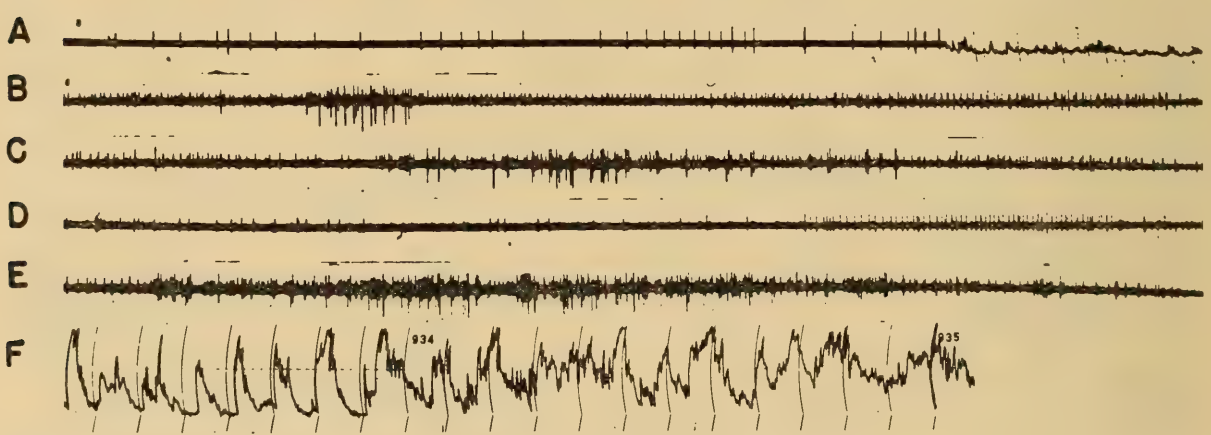

Figure 2. A. Oscilloscope record of impulses on the phallic nerve of a normal cockroach, followed by a polygraph record showing the integrated electrical activity from the same nerve. B–E. Oscilloscope records from the same nerve. The roach had been decapitated twenty minutes before the record was made. Note the increased activity. F. Polygraph record of integrated nerve impulses from the phallic nerve of the same roach taken immediately after B–E. The film speed for the oscilloscope record was 25 cm./sec.; the polygraph tape moved 2.5 mm./sec.

thirty minutes (Fig. 2). Individual efferent fibers appear to follow their own burst frequency, giving a rhythmic and often complex pattern to the activity in the compound nerve. In most experiments after thirty minutes the burst pattern usually reaches its full development and remains constant for some hours. Occasional silent periods have been observed, however.

When an active extract of corpora cardiaca is applied to the nerve cord or injected into the head of a roach with an intact nerve cord, the effects of nerve cord transection or decapitation are mimicked (Fig. 3; compare with Fig. 3C, Roeder, Tozian and Weiant, 1960). The sequence of changes in the phallic nerve activity follows the same time course in the gradual development of bursts, and the magnitude of the response is very similar to that released by cord transection. The initial time lag when extract is applied is similar to that seen with decapitation. Desheathing portions of the abdominal ganglia and cord does not decrease this interval. The

only consistent difference is that activity produced by the corpus cardiacum extract is temporary, declining after about one hour. The bursting rhythm becomes irregular and the activity slowly dies down to its original level. If extract is reapplied to the cord every twenty minutes or so, the rhythmic efferent bursts may be maintained for several hours. These effects were observed in about sixty preparations.

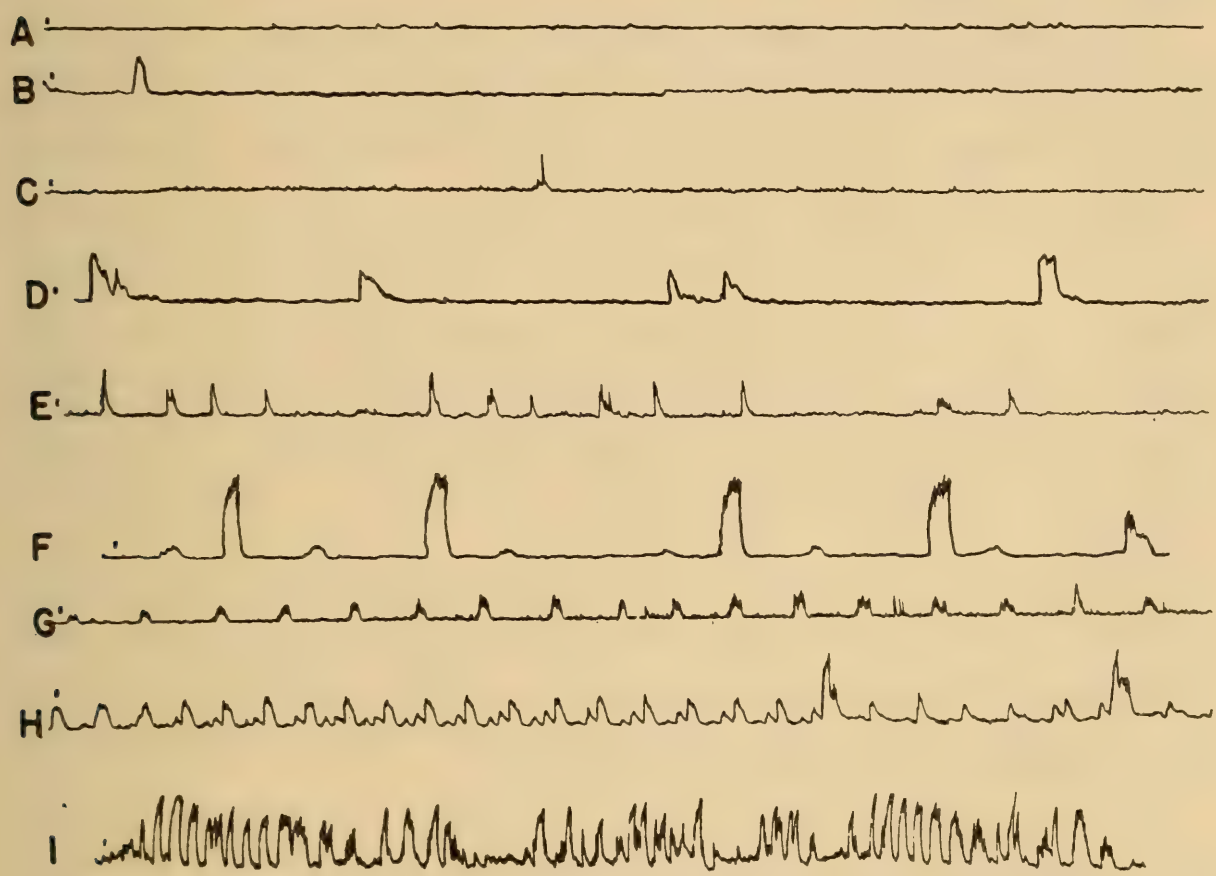

FIGURE 3. Polygraph records showing the integrated phallic nerve activity from the following roaches: A and B. Normal cockroaches. C. Roach with abdominal nerve cord exposed to corpus cardiacum extract, ½ pair/0.01 ml., for 30 minutes (concentration below threshold). D. Roach with abdominal nerve cord exposed for 25 minutes to corpus cardiacum extract, 3 pairs/0.01 ml. from penultimate stage nymphs. E. Roach with abdominal nerve cord exposed to corpus cardiacum extract, 1 pair/0.01 ml., for 30 minutes. F. Roach with abdominal nerve cord exposed to corpus cardiacum extract, 2 pairs/0.01 ml. for seven minutes and (G) for 30 minutes. H. Roach which had had 3 pairs of corpora cardiaca in 0.02 ml. of saline injected into its head capsule 30 minutes before. I. Roach which had had 4 pairs of corpora cardiaca in 0.02 ml. of saline injected into its head capsule 35 minutes before.

The effects of extract concentration. The concentration of corpus cardiacum material necessary to produce the efferent nerve bursts varies both with the size and the species of the donor roaches and with the sensitivity of the test preparation. In general, the larger the adult size of the species, the more active were the extracts of its glands. In terms of their activity when tested on *Periplaneta,* extracts of *Blaberus giganteus* and *Blaberus craniifer* were about equally potent, while the glands of other species ranged down in activity in the following order: *Leucophaea maderae, Periplaneta americana, Byrsotria fumigata,* and *Diploptera punctata.*

The extract is always diluted by the blood of the roach so that quantitative information is difficult to obtain. An extract made from three or four pairs of corpora cardiaca diluted with 0.01 or 0.02 ml. of saline and injected into the head capsule produces pronounced increases in efferent fiber activity in the phallic nerves followed by rhythmic bursts of spikes. Extracts containing one or more pairs of corpora cardiaca per 0.01 ml. of saline when applied to the abdominal cord have similar effects (Fig. 3).

Control extracts were made from abdominal nerve cord tissue and applied in the same manner to the head ganglia and abdominal nerve cords of test roaches. Abdominal cord extract caused occasional transient increases in the activity of both the abdominal cord and the efferent nerves, but the regular and continuing efferent nerve spike bursts were never seen.

In experiments employing more concentrated extracts, containing up to three and a half pairs of corpora cardiaca per 0.01 ml., the initial time lag was unaffected, but the level of phallic motor nerve activity rose more rapidly than with less concentrated extracts and the final frequency attained by the efferent nerve spike bursts was higher. These rapid bursts, when integrated, appeared on the polygraph with a lower amplitude and a shorter duration than the slower-frequency bursts obtained with the less concentrated extracts (Fig. 3, F and G). The burst frequency about thirty-five minutes after the first application of extract provides a rough measure of the potency of the extract.

In four out of seven experiments in which concentrated extracts containing three and one-half pairs of glands per 0.01 ml. were applied to the abdominal cord, the phallic motor nerve activity was blocked after twenty to thirty minutes. This block was only partially reversible by washing. In fact, all attempts to wash out the effects of the corpus cardiacum extracts met with little success if the extract had been on the preparation for fifteen minutes or more. This may have been due in part to the difficulty of irrigating these preparations.

Activity in the abdominal nerve cord. The experiments of Özbas and Hodgson (1958) showed that nerve impulses in isolated roach abdominal nerve cords were blocked when the cords were immersed in extracts of corpora cardiaca. We have confirmed these observations and have also noticed a short period of excitation which seems to precede the block in such isolated cords. It has proved extremely difficult to block nerve impulses in abdominal cords with these extracts if the cords remain *in situ* with the cercal afferents intact and the head and thoracic portions of the cord still attached. When applied to a preparation in which the central nervous system is almost undamaged, the extracts usually cause a brief increase in the level of activity in the abdominal connectives. A tendency for some nerve cells to fire short trains of impulses becomes apparent after the extract has been on the cord twenty to thirty minutes. A period of depressed activity sometimes follows the period of excitation.

Simultaneous recordings made from abdominal connectives and from the phallic nerve do not reveal reciprocal changes in the levels of activity of the cord connectives and the motor nerves after decapitation or after application of extracts containing one to three pairs of corpora cardiaca per 0.01 ml. As the efferent nerve activity increases and bursts of nerve spikes begin to appear on the phallic nerves, the cord activity increases for a time and then usually returns to its previous

level. The cord appears depressed only when concentrations of extracts higher than three pairs per 0.01 ml. were used.

Nerve activity in the thoracic region. The changes induced by corpus cardiacum extracts in the activity of nerve 3 from the metathoracic ganglion and in the thoracic connectives were somewhat more complex than those obtained in the abdominal region. Of twenty-three experiments, fourteen showed increased activity followed by bursts similar to those observed in the phallic nerves. The change occurred twelve to twenty minutes after the extract was applied. In four cases the activity decreased and in four the efferent impulses were blocked. In these cases where the extract caused a temporary or partial block in cord and efferent nerves, twenty minutes were allowed for recovery, but the activity did not return to normal. However, if these roaches were then decapitated, there was an increase in the frequency of the active fibers in both the thoracic cord and nerve 3, and in one case there was also an increase in the number of fibers firing in nerve 3.

In one experiment there appeared to be no change in activity. A temporary decline or cessation of efferent activity was also noted at one period in four of the thirteen preparations which showed an over-all increase in activity and bursts. These changes induced by corpus cardiacum extract correspond closely with those following decapitation or transection of the thoracic connectives (Weiant, 1958).

Changes in activity recorded from electrodes placed on the nerve cord followed very closely the changes in nerve 3 described above. It seems probable that the increased efferent activity was picked up by the cord electrodes, and that this would have masked any decreased activity that might have occurred in the internuncial fibers within the connectives.

In a few cases where the extract reached the exposed thoracic tissues, violent tremors developed in the flight muscles. Since all motor branches from the thoracic ganglia were severed, this suggests that the extract has a direct action on the thoracic muscles.

Abdominal pulsations often coincided with and obscured the thoracic nerve activity. If the abdomen was cut off at this time, there was some decline in the activity in nerve 3. However, this operation rarely brought the activity down to the level recorded previous to the application of extract. If severing the abdomen was followed by decapitation, a slight increase in the frequency of the activity in nerve 3 occurred.

Synaptic conduction. Six experiments were performed using the classic cercal nerve-giant fiber preparation (Roeder, Kennedy and Samson, 1947) to determine the effects of corpus cardiacum extracts on a sensory synapse. Extracts containing one to three pairs of glands per 0.01 ml. were applied or injected and left on the preparation for thirty to forty minutes. The only change which was observed was a slight lowering of the electrical threshold of the synapse in every case.

Comparison with DDT-toxin. Sternberg and Kearns (1952) have reported the presence of an excitor substance liberated from the nervous system of roaches poisoned with DDT. Four attempts have been made to collect this substance using a technique employed by Sternberg. The abdominal cord activity increased greatly after exposure to the saline containing the substance and many impulse trains were seen. No rhythmic spike bursts were observed on the phallic nerves,

however. It was concluded that the DDT-toxin was not the same as the principle in the corpus cardiacum extracts which causes phallic efferent nerve bursts.

Conclusions

The foregoing experiments demonstrate that, except for its reversible action, the extract of corpora cardiaca taken from several species of roaches mimics, in the American roach, removal of the inhibitory system as postulated by Roeder, Tozian and Weiant (1960). The simplest interpretation is that the corpus cardiacum extract suppresses the action of an inhibitory system, there being no evidence that it has any action on excitatory synapses.

If this is the case, a decrease in the activity of those fibers within the nerve cord that mediate the inhibition must occur in the presence of extracts of corpora cardiaca. This could not be detected by electrodes placed in contact with the outside of the nerve cord, although in the abdominal preparations the extract-induced increases in cord activity were transitory and insignificant compared with those occurring in the efferent fibers. Perhaps this is to be expected since the fibers comprising the inhibitory system may occupy only a small portion of the cord substance (Hess, 1958), and from the aspect of an external electrode they are probably shunted by many other fibers either unaffected by, or even released by, the blockage of the inhibitory system.

One perplexing observation in the earlier work (Roeder, Tozian and Weiant, 1960) was that the descending inhibitory system could not be blocked by KCl or by anelectrotonus, the only way of blocking it at that time being by cord transection. To this may be added the current observation that the inhibitory system may be inactivated not only by applying corpus cardiacum extract to its center or point of origin in the subesophageal ganglion, but also anywhere along its path from the head to the locally-originating neurones. It is difficult to visualize a classical nerve pathway operating in this manner, although no alternative mechanism can yet be proposed.

Summary

1. Increased activity and regular bursts of efferent nerve spikes are seen on nerves IX and X from the cercal ganglion of roaches when extracts of corpora cardiaca are applied to the exposed abdominal cord or injected into the head capsule. Such motor nerve activity is usually inhibited in the intact animal by the presence of some influence arising in the subesophageal ganglion (Roeder, Tozian and Weiant, 1960). The corpus cardiacum extracts appear to suppress the action of this inhibitory center. The threshold concentration for this effect is approximately one pair of corpora cardiaca per 0.01 ml. of saline when the extract is applied to the abdominal cord, or three pairs injected into the head in about 0.01 to 0.02 ml. of saline. In decapitated male mantids a similar type of nerve fiber activity is believed to result in behavior associated with mating.

2. The corpus cardiacum extracts seem temporarily to increase the activity of the abdominal nerve cord in preparations with an intact central nervous system. When high concentrations of extract are used, the cord activity is occasionally depressed or blocked. The effects of the extract are only partially reversible by washing the preparation with saline.

3. The activity of certain efferent fibers in the thoracic region is similarly affected by corpus cardiacum extract, the action mimicking that produced by decapitation or cord transection.

4. Corpus cardiacum extracts have no significant effects on the transmission of impulses at the synapse between the cercal nerve and the giant fibers in an electrically stimulated preparation.

5. The DDT-toxin of Sternberg does not have the same effect on the roach nervous system as the active principle in extracts of corpora cardiaca.

6. The corpus cardiacum extracts may be preserved successfully by quick-freezing. Their activity is decreased by prolonged heating, but warming for five minutes at 90° to 100° C. leaves the potency unaffected. Such warming seems to increase by several hours the length of time that an extract will remain potent.

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